Text by Ben Short
bshort@rockefeller.edu

Actin oligomers hit the assembly line

Study identifies a new way for actin filaments to polymerize in vivo.

Actin filament production is a "green" industry. New filaments are assembled from a renewable pool of actin monomers, recycled from older, depolymerizing filaments. Okreglak and Drubin now reveal that old filaments aren't always completely broken down—short fragments generated by the severing protein cofilin are reused directly to construct new actin structures (1).

Voytek Okreglak and David Drubin, from the University of California, Berkeley, study actin dynamics at sites of endocytosis in budding yeast. "Endocytosis culminates in a predictable burst of actin polymerization followed by rapid disassembly, so we can watch and analyze the process at the cell cortex," Drubin explains. To make these dynamics easier to see, the researchers often use mutant yeast that grow particularly large actin tails at their endocytic sites. In these enlarged tails, actin subunits are added at the end nearest the plasma membrane and removed at the opposite end, leading to a measurable flux of actin toward the cell interior. "It's similar to the leading edge of a migrating fibroblast," says Drubin.

In 2007, Okreglak and Drubin used this system to study the filament-severing protein cofilin's contribution to actin turnover in endocytosis (2). Okreglak followed this up by examining Aip1, a protein that works with cofilin to disassemble actin filaments in vitro (3). To his surprise, deleting *Aip1* had no effect on disassembly rates in vivo, but in the course of his studies Okreglak noticed something even odder: actin filaments continued to assemble at endocytic sites in the presence of high concentrations of latrunculin A (lat A), a drug that inhibits polymerization by sequestering actin monomers. This lat A–resistant assembly also occurred in wild-type cells, but was more pronounced in yeast lacking Aip1. "This was totally unexpected," says Drubin. "It meant that there was some property of actin that we didn't understand."

Focal Point

David Drubin and Voytek Okreglak (holding his son, Simon) discover that actin filaments can assemble through the direct annealing of short actin fragments, rather than relying exclusively on the addition of monomers. The pathway is revealed by the continued assembly of actin (red) at sites of endocytosis (green) in the presence of latrunculin A (bottom), a drug that inhibits the polymerization of actin monomers. The process is particularly evident in budding yeast lacking the protein Aip1 (pictured), which, the researchers show, converts the actin oligomers produced by the filament-severing protein cofilin into latrunculin A–sensitive monomers.

One possibility was that these actin filaments evaded lat A inhibition by directly incorporating short actin oligomers instead of monomers. This mode of assembly has been seen in vitro (4, 5) but it wasn't thought to occur in living cells—actin assembly is a highly controlled process, and it's not clear how oligomers would be regulated. "Also," adds Drubin, "we assumed that actin disassembly always went to completion, so that there wouldn't be a pool of actin oligomers available in vivo."

> "Oligomers have been overlooked, but they clearly contribute to actin assembly."

If lat A–resistant actin polymerization is mediated by oligomer assembly, why is the process enhanced in cells lacking Aip1? The researchers wondered whether Aip1 regulates the balance between actin oligomers and monomers. The protein's function in actin disassembly could be to generate monomers from the oligomeric fragments produced by cofilin. In Aip1's absence, the fragments wouldn't be dismantled, and would be more available for lat A–resistant filament assembly.

To test this idea, Okreglak and Drubin examined how Aip1 affected the oligomeric state of actin. In vitro, Aip1 increased the amount of monomeric actin produced when actin filaments were broken down by cofilin. In yeast cells lacking Aip1, on the other hand, the amount of actin monomer was reduced by 45%.

Thus, Aip1 renews the actin monomer pool during filament disassembly, but this regeneration isn't necessary because short actin oligomers can be used to build new filaments instead. "Oligomers have really been overlooked," says Drubin. "But they clearly contribute to actin assembly at endocytic sites, even in wild-type cells."

Drubin now plans to investigate how Aip1 separates oligomeric fragments into monomers, and to determine how oligomers are incorporated into growing filaments at endocytic sites. "No one had detected this pathway before," says Drubin. "Now people have to look to see if it's involved in other actin assembly processes too."

1. Okreglak, V. and D.G. Drubin. 2010. *J. Cell Biol.* doi:10.1083/jcb.200909176.
2. Okreglak, V., and D.G. Drubin. 2007. *J. Cell Biol.* 178:1251–1264.
3. Rodal, A.A., et al. 1999. *J. Cell Biol.* 145:1251–1264.
4. Kawamura, M., and K. Maruyama. 1970. *J. Biochem.* 67:437–457.
5. Murphy, D.B., et al. 1988. *J. Cell Biol.* 106:1947–1954.